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Grau de Química

Treball Final de Grau

Analysis of organophosphorus flame retardants in marine biota
Anàlisi de retardants de flama organofosforats en biota marina

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En primer lloc agrair a tots els membres del CSIC per donar-me l'oportunitat de dur a terme aquest projecte al centre, principalment a la Dra. Ethel Eljarrat per ajudar en tot moment que ha fet falta. També a la doctorand Berta Sala que m'ha ensenyat la metodologia de treball i m'ha ajudat sempre que ha fet falta, tenint molta paciència amb mi. Finalment també vull agrair a la Dra. Anna Rigol, tutora d'aquest projecte, la qual m'ha aconsellat i també m'ha guiat per tal de no perdre el ritme.

No oblidar-me de tota la gent que he conegut del grup IDAEA-CSIC que han sigut molt amables tant en les estones de treball al laboratori com en els moments de descans, fent-me sentir còmode en tots els moments que hem estat junts.

REPORT

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1. SUMMARY

Flame retardants (FRs) are chemicals added to materials in order to slow or prevent the start or growth of fire. The use of organophosphorus FRs (OPFRs) has increased the last years because the use of polybrominated diphenyl ethers (PBDEs), that are other type of FR, is currently banned. OPFRs can substitute PBDEs because of their technical characteristics, and they are also used as plasticizers and anti-foaming agents. Although there is controversy about their toxicity, there can be found some general toxicological effects of the OPFRs: reproductive and developmental toxicities, association with neurotoxicity, endocrine disruption and carcinogenicity. This project will focus on the analysis of 19 OPFRs in biota samples of environmental interest as bioindicators, specifically in sea turtles and marine fish. The analytical method is based on turbulent flow chromatography (TFC) in combination with high pressure liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS-MS). The extraction was performed by ultrasound liquid extraction, and purification was done with the TFC system. This method has allowed the evaluation of OPFRs in turtle and fish samples archiving satisfactory results. OPFRs were detected in all analysed samples, showing their ubiquity in the environment as well as their capacity for bioaccumulation.

Keywords: organophosphorus flame retardants, plasticizers, marine biota, high performance liquid chromatography, tandem mass spectrometry, turbulent flow chromatography.

2. RESUM

Els retardants de flama (FRs) són productes químics que s'afegeixen a materials per tal de retardar o prevenir l'inici o el creixement d'un foc. L'ús dels FRs organofosforats (OPFRs) ha incrementat degut a que l'ús dels èters difenílics polibromats (PBDEs), els quals són un altre tipus de FRs, s'ha prohibit. Els OPFRs poden substituir als PBDEs degut a les seves característiques tècniques, i també es fan servir com a plastificants i agents antiespumants. Tot i que la seva toxicitat no està ben definida, hi ha alguns efectes tòxics generals dels OPFRs: toxicitat reproductiva i en el desenvolupament, associació amb la neurotoxicitat, disrupció endocrina i carcinogenicitat. Aquest projecte es centrarà en l'anàlisi de 19 OPFRs en mostres de biota d'interès ambiental a mode de bioindicadors, concretament en tortugues i peixos marins. El mètode analític està basat en una cromatografia de flux turbulent (TFC) combinada amb cromatografia líquida d'alta resolució (HPLC) acoblada a espectrometria de masses en tàndem (MS-MS). L'extracció es va realitzar en fase líquida fent servir ultrasons, i la purificació es va fer amb el sistema TFC. Aquest mètode ha permès l'avaluació dels OPFRs en mostres de tortugues i peixos obtenint resultats satisfactoris. Els OPFRs es van detectar a totes les mostres analitzades, demostrant que es troben presents en el medi ambient i també la seva capacitat de bioacumulació.

Paraules clau: retardants de flama organofosforats, plastificants, biota marina, cromatografia líquida d'alta resolució, espectrometria de masses en tàndem, cromatografia de flux turbulent.

3. INTRODUCTION

3.1. FLAME RETARDANTS

Due to the growth of the polymer industry over the past 50 years, the number of polymers with different properties and applications has increased. This makes that polymers can be found in clothing and furniture, electronics, vehicles, computers and other fields. These polymers use to be petroleum-based, meaning that they are flammable, and as some final products must meet fire safety regulations, flame retardants (FRs) are applied to them, also they are applied to other materials like plastics, woods or paper.

FRs are added to materials to slow or prevent the start or growth of fire. Its usage has increased as different industries consume more polymeric materials to supply the demand. There can be classified in four major groups, which are: inorganic, halogenated (brominated and chlorinated), organophosphorus and nitrogen-based FRs.

The mode of action of FRs is related with the combustion reaction, a gas phase reaction that involves a fuel source and oxygen with four steps: preheating, volatilization/decomposition, combustion and propagation. FRs can act at any of these steps from the combustion process and inhibit it or prevent the occurrence, depending on the FR behaviour [1].

3.1.1. Problems of FRs

FRs can get into different medias as air, water or soil during manufacture, but also once the final product is done these chemicals can be leaked. E-waste is another important source of FR when it processed (burned or dismantled), and once these medias are polluted, people and animals are exposed to FRs [2].

Even if FRs offer benefits to the materials where they are added, there are evidences showing that a big part of them have adverse health effects in animals and humans. Some of these effects are: endocrine and thyroid disruption, reproductive toxicity, adverse effects on fetal and child development and neurologic function, cancer and impacts to the immune system [2].

Polybrominated diphenyl ethers (PBDEs) are a type of halogenated FRs that its persistence, long-range atmospheric transport, bioaccumulation and toxicity has been confirmed [3]. Due to this, its usage has been banned, generating a new demand for other type of FRs [4].

3.2. ORGANOPHOSPHORUS FRS

Organophosphorus FRs (OPFRs) can substitute brominated FRs because of its technical characteristics. They are also used as plasticizers and anti-foaming agents in many different industries, as plastics, textile, furniture, construction, electronics, vehicle or petroleum.

Most of the OPFRs are added to the materials as additives instead of being chemically bonded. This produces an easier release to the environment via volatilization, abrasion or leaching, this is a fact as different OPFRs have been reported in some environmental matrices as dust, air, water, sediment, soil and biota samples [3].

3.2.1. Classification

The OPFRs can be divided in three groups depending on its general structure: the phosphinates, the phosphonates and the phosphate esters (*Figure 1*). The halogenated OPFRs is a widely used group because it combines the properties of phosphorus and halogen components. The halogen decreases the mobility in the polymer, producing an increase of the lifetime in the final product [5].

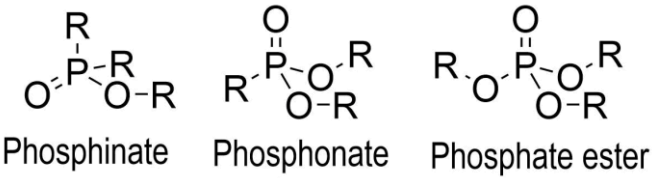


Figure 1. General structures of OPFRs.

There is also other classification apart from these three groups, which gives to two types of OPFRs, first of them are the reactive ones that are chemically build into the polymer molecule, avoiding the loss of the OPFR in the lifetime of the product. The second group are the additive FRs, that are mixed into the polymer but without chemical binding, which makes that the concentration of them can decrease during the lifetime of the final product, releasing them to the environment and decreasing the flame retardancy properties [5].

The compounds that will be studied are summarized in *Table 1*, classifying them on the previously mentioned classification.

Table 1. Target compounds.

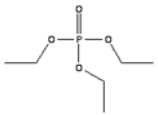
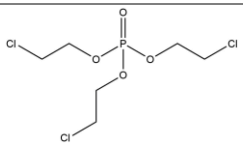
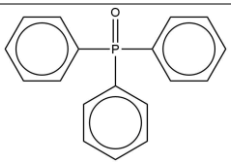
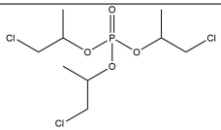
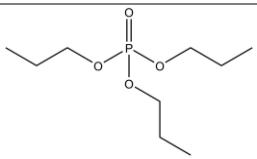
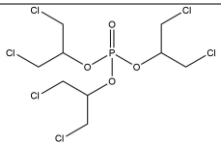
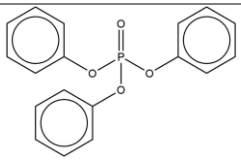
Acronym	Chemical name	Chemical structure	OPFR group
TEP	triethyl phosphate		Phosphate ester
TCEP	*tris(2-chloroethyl)phosphate*		Halogenated phosphate ester
TPPO	*triphenylphosphine oxide*		Phosphinate
TCIPP	*tris(2-chloroisopropyl) phosphate*		Halogenated phosphate ester
TPP	*Tripropyl phosphate*		Phosphate ester
TDCIPP	*tris(1,3-dichloro-2-propyl) phosphate*		Halogenated phosphate ester
TPHP	*triphenyl phosphate*		Phosphate ester

Table 1 (*continued*). Target compounds.

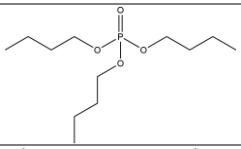
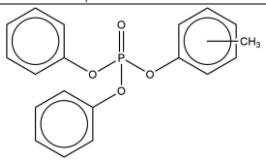
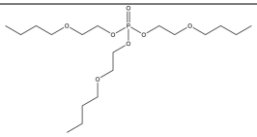
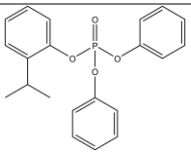
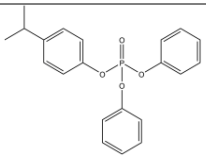
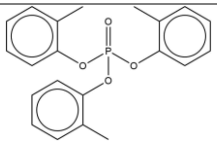
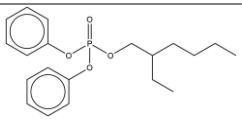
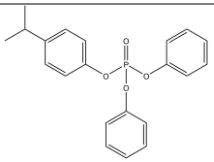
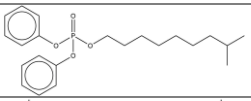
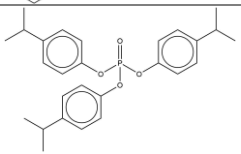
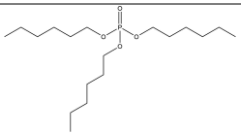
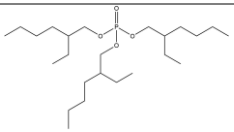
Acronym	Chemical name	Chemical structure	OPFR group
TNBP	*tributyl phosphate*		Phosphate ester
DCP	*diphenyl cresyl phosphate*		Phosphate ester
TBOEP	*tris(2-butoxyethyl) phosphate*		Phosphate ester
2IPPDPP	*2-isopropylphenyl diphenyl phosphate*		Phosphate ester
4IPPDPP	*4-isopropylphenyl diphenyl phosphate*		Phosphate ester
TMCP	*tricresyl phosphate*		Phosphate ester
EHDP	*2-ethylhexyldiphenyl phosphate*		Phosphate ester
B4IPPPP	*Bis(4-isopropylphenyl) phenyl phosphate*		Phosphate ester

Table 1 (*continued*). Target compounds.

Acronym	Chemical name	Chemical structure	OPFR group
IDPP	*isodecyldiphenyl phosphate*		Phosphate ester
IPPP	*Isopropyl phenyl phosphate*		Phosphate ester
THP	*trihexyl phosphate*		Phosphate ester
TEHP	*tris(2-ethylhexyl) phosphate*		Phosphate ester

3.2.2. Mechanisms of flame retarding

There are many different mechanisms for FR to prevent fire, depending on its type. For the OPFRs there is not a single way to act as FR. The halogenated OPFRs act in the gas phase by removing the H^+ and OH^- radicals from the flammable gases, which react with the Br or Cl atoms, resulting in a slow of the burning process and a reduction of the spreading of the fire. The non-halogenated act in the solid phase instead of the gas phase. The mechanism is based in the reaction that phosphorus has when it is heated. It forms a polymeric form of phosphoric acid, causing a char layer that shields the material from oxygen, preventing the formation of flammable gasses [5].

3.2.3. Toxicity

There is controversy about the toxicity of the OPFRs. There can be found reports giving different information about a same compound. For example, in the TPHP there are reports where it is said that is possible associated with delayed neurotoxicity, other one mention low

neurotoxicity and a last one found no evidence of the compound causing neurotoxicity in animal experiments [3].

Even if there is controversy, there can be found some general toxicological effects of the OPFRs as: reproductive and developmental toxicities, association with neurotoxicity, endocrine disruption and carcinogenicity. These toxicity problems related to the OPFRs made that the use of some of them has been restricted, and this could show that they are not the best substitute for halogenated FRs, which have been more studied, and its toxicity is well known [6].

3.3. OPFRs IN AQUATIC BIOTA

3.3.1. Bioaccumulation and biomagnification

There are studies where OPFRs show an accumulative potential that might vary between different species and individuals. One of them found that there were variations between fishes from the same sampling site, where perch of different sizes have different concentrations of OPFRs. The larger perches had higher concentrations that the smaller ones, and this occur in different sampling sites, suggesting the bioaccumulation of OPFRs [7]. But there are some OPFRs whose affinity for lipids is limited in fishes, which might suggest that the accumulation is not just associated with the lipid content, as some prevalent OPFRs have been detected in organisms from aquatic environments even if its bioaccumulation factor is low [8].

The biomagnification factors have been calculated for OPFRs in aquatic food webs, and there has been found that some of them present biomagnification but other ones do not show biomagnification. The results are not enough to determine a clear understanding of the biomagnification in ecosystems, generating a need of more investigations in this field, as well as in the mechanism of biomagnification of OPFRs as there is not yet a clear comprehension of it [8].

3.3.2. Biota levels

There are scarce published works that show the OPFRs levels in biota samples, there can be found in organisms as fishes or marine mammals. These studies can give an idea about the levels that can be found in similar biota samples, even if they are not exactly the ones that will be analysed. In the case of the turtles there are studies of persistent organic pollutants (POPs) such as dichlorodiphenyltrichloroethane and its main metabolites (∑DDTs), polychlorinated

biphenyls (∑PCBs), chlordanes (∑CHLs), polybrominated diphenylethers (PBDEs), dichlorodiphenyldichloroethylene (DDE), Mirex and hexachlorobenzene (HCB) [9], or other ones as ∑PCBs, ∑DDTs and perfluorinated compounds (∑PFCs) [10]. But there cannot be found works that study the content of OPFRs in turtles.

There is a study in fish samples from three different European river basins: Evrotas, Adige and Sava. The results of ∑OPFRs are significantly different between the three different river basins. In Evrotas river basin there were found concentrations between 34.1-55.5 ng/g lipid weight (lw) (mean of 40.1 ng/g lw), in Adige river basin the concentrations were the highest being between 50.5-650 ng/g lw (mean of 286 ng/g lw), and finally in Sava river basin the concentrations were between 14.4-196 ng/g lw (mean of 84 ng/g lw) [11].

Also, there are studies in dolphins where the OPFRs are analysed in different tissues, giving different results depending on the type of tissue. The tissue of main interest for the current study is the muscle because the kind of sample that will be studied is muscle from turtle and fish. In the muscle tissue there were found levels of ∑OPFRs between 69.5-2939 ng/g lw (mean of 645 ng/g lw), but it was not the tissue with a highest concentration in dry weight (dw), bubbler and brain have a higher mean of ∑OPFRs without converting it to lw [12].

3.3.3. Impact on sea turtles

Sea turtles are prone to eat buoyant debris that can be found in the seas and oceans, as plastic pieces and other kind of rubble that has a high potential of contain OPFRs. This gives problems to the survival possibilities for these turtles, because apart of eating debris they can get trapped in some of them, mainly in the initial development stages as they happen in the open sea, where the rubble can be accumulated.

This generates an interest to study the amount of OPFRs in the turtles as they are prone to eat the plastic scraps, there are more possibilities to get positive results in the analysis of OPFRs in her organism [13].

4. OBJECTIVES

The main objective in this work is to assess the impact of OPFRs in the marine environment using biota samples as bioindicators. To archive this goal, the following specific objectives were established:

· Improvement of a previous developed method of analysis of OPFRs by including five new compounds.

· Determination of the concentration levels of 19 different OPFRs in sea turtles, to assess the effect of plastic debris in the presence of OPFRs in turtle tissues . These compounds have not been studied yet in turtles. However, as plastic debris appears in turtles, this could cause the presence of plasticizers such as OPFRs in their tissues.

· Comparison of OPFR concentration levels in the same species of turtle from two different locations.

· Determination of the concentration levels of OPFRs in marine fish, as most studies of OPFRs in fish samples are from freshwater fish.

5. EXPERIMENTAL SECTION

5.1. MATERIALS AND INSTRUMENTATION

5.1.1. Standards and chemicals

As standards were used:

· Tris(2-chloroethyl)-phosphate (TCEP), tris(2-butoxyethyl) phosphate (TBOEP), trihexyl phosphate (THP), tris(2-ethylhexyl) phosphate (TEHP) and tris(chloroisopropyl)-phosphate (TClPP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

· Isopropyl phenyl phosphate (IPPP) was purchased from Chiron (Trondheim, Norway).

· 2-ethylhexyldiphenyl phosphate (EHDPP) and isodecyldiphenyl phosphate (IDPP) were purchased from AccuStandard (New Haven, CT, USA).

· Tricresyl phosphate (TMCP) was purchased from Dr. Ehrenstorfer (Augsburg, Germany).

· Diphenyl cresyl phosphate (DCP), triphenyl phosphate (TPHP), tris(1,3-dichloro-2-propyl) phosphate (TDClPP), tributyl phosphate (TNBP), triphenylphosphine oxide (TPPO), triethyl phosphate (TEP) and tri-n-propyl phosphate (TPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

· 2-isopropylphenyl diphenyl phosphate (2IPPDPP), 4-isopropylphenyl diphenyl phosphate (4IPPDPP) and bis(4-isopropylphenyl) phenyl phosphate (B4IPPPP) were purchased from Wellington Lab-oratories Inc. (Guelph, ON, Canada)

As internal standards were used:

· d15-TPHP was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

· d27-TNBP, d15-TDClPP, 13C2-TBOEP and d12-TCEP were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada).

As reagents for the extraction and work-up:

· Acetone and hexane solvents for organic trace analysis were purchased from J.T. Baker (Center Valley, PA, USA).

· Methanol and water solvent were obtained from Merck (Darmstadt, Germany).

5.1.2. Instrumentation and equipment

- Centrifuge 5810 R of Eppendorf Ibérica (Spain).

- Nitrogen evaporators: TurvoVap LV of Caliper LifeSciences (Hopkinton, USA).

- Turbulent flow chromatograph TurboFlowTM system from Thermo Scientifics. With the columns: CyclonTM-P (0.5 x 50 mm) and C18-XL (0.5 x 50 mm) for purification and Purosphere Star RP-18 (125 mm x 0.2 mm) for analytical separation, all of them purchased from Thermo Scientifics.

5.2. SAMPLES

5.2.1. Turtle

The turtle samples that were analysed are from loggerhead sea turtle (Caretta caretta) (*Figure 2*), which is a species of oceanic turtle that can be found in almost all the seas and oceans. Depending on its life cycle they can be found in different zones as open sea, coastal areas, bays or estuaries. They live in the Pacific, Atlantic or Mediterranean, and in this case the analysed turtles are from the Mediterranean Sea, from two different zones, the Catalan and the Balearic coasts [14].

Figure 2. *Caretta caretta*.
(*Strobilomyces, 29/05/2019 via Wikimedia Commons, Creative Commons attribution*)

The samples are from turtle muscle, and they were collected between 2014 and 2017 in the places previously mentioned. In order to make the results more comparable, all the samples were turtles which curve carapace length (CCL) was more or less between 40cm and 60cm (*Appendix 1, Table A*). The turtles with this size use to be juvenile individuals, which have between 6 and 12 years old (a turtle of this specie can live around 60 years).

The total of samples from turtle is 42, each of them corresponding to different individuals. 20 of them are from Catalan coasts, and the other 22 correspond to the ones from Balearic coasts. The samples were previously lyophilized by other members from IDAEA-CSIC to let them prepared for the extraction process.

5.2.2. Fish

The fish samples that were analysed are from a tuna commonly known as little tunny (Euthynnus alletteratus). It is a tuna which size is relatively small, in the Mediterranean the maximum size is around 100cm of fork length (FL) and about 12kg of weight (*Figure 3*) [15].

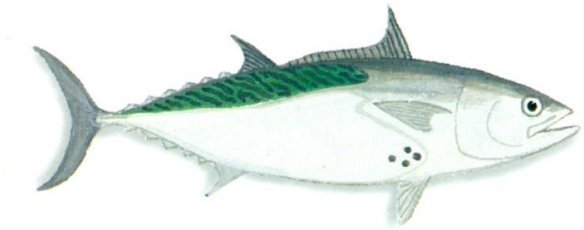


Figure 3: *Euthynnus alletteratus*.
(*Xavier Romero-Frias, 29/05/2019 via Wikimedia Commons, Creative Commons attribution*)

In this case the sampling was done in La Azohía (Cartagena, Spain), the days 30 and 31 of May of 2016, and there is a total of 14 samples from different individuals. The weight of these samples is not homogeneous, it is specified with the sex of each individual in *Appendix 1* (*Table B*). In this case the sample is from liver instead of muscle.

The samples were lyophilized previously to arrive to IDAEA-CSIC, but they were in plastic vials, which might contaminate them. To consider this possibility hydromatrix was added to

empty vials as the ones where the samples were stored, in order to analyse them and know if the contamination can be attributed to the vial.

5.3. EXTRACTION

The method that has been followed for this extraction is the one that appears in the article from Giulivo, M., et al. [16], which has been previously optimized by the group of IDAEA-CSIC for fish samples, and it will be used for fish and turtle samples. The whole process is resumed in *Figure 4*.

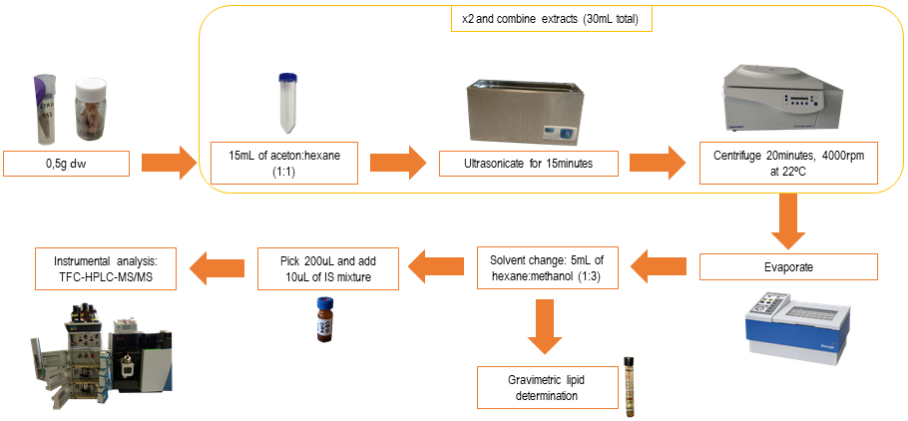


Figure 4. Process scheme.

An ultrasound extraction was performed to 0.5g dw of sample, using as solvent 15mL of a hexane:acetone mixture (1:1). To carry out this extraction the tubes containing the sample and the solvent were sonicated for 15 minutes and, after this, centrifugated for 20 minutes at 4000 RPM and 22ºC. This extraction was performed twice for each sample, and both extracts were combined and evaporated using a purified nitrogen stream in TurvoVap LV to a small volume and transferred to a centrifuge tube (previously tared). In this centrifuge tube the solvent was completely removed using the purified nitrogen steam in TurvoVap LV to change the solvent.

Samples were reconstituted by adding 5mL of a hexane:methanol mixture (1:3). After this the samples were sonicated during few seconds and finally centrifuged for 10 min at 4000 RPM and 18ºC. From this final solution an aliquot (200 µL) was transferred to a vial, and 10 µL from a mixture of the internal standard (IS) were added to these 200 µL. The mixture had a concentration of 1 ng/µL of: d12-TCEP, d15-TDCIPP, d15-TPHP, d27-TNBP and 13C2-TBOEP.

Once the IS was added to the vial, the solution was ready for analysis. Two aliquots of 200 µL were taken to prevent the possible risk of losing the sample, but the IS was just added to the one that was going to be analysed.

With the resultant 4.6mL of extracts solution, a determination of the lipidic content that has been extracted was done by gravimetric analysis. To do this the solution was evaporated to dryness using a purified nitrogen steam in TurvoVap LV and once the solvent was removed the tube was left in the oven for 1h 30', and afterwards weighted, then left again in the oven for 30' and weighted again after this time. The process was repeated until a constant weight was achieved.

5.4. OPTIMIZATION OF MASS SPECTROMETRIC DETECTION FOR NEW COMPOUNDS

In order to increase the amount of analysed OPFRs, the analysis of 5 new compounds, that were not included, in the previous method, was optimized [16]. These compounds won't be considered as quantitative results because the recoveries tests were not performed. It will be considered that the extraction works as for the previously optimized OPFRs. These new compounds are: 2IPPDPP, 4IPPDPP, B4IPPPP, TEP and TnPP.

To optimize MS-Ms analysis of the compounds, a direct injection was done to the tandem mass spectrometer to select the two most intense transitions, in order to be able to analyse these compounds. The spectrometric conditions were the same used at the method [16], the one that was changed was the spray voltage. Depending on the obtained mass spectrum, the spray voltage was chosen positive (3600V) or negative (-2500V). In all the 5 compounds the positive spray voltage was chosen because the spectrum was clearer.

Once the voltage was chosen the precursor ion mass was checked. In some cases, its signal appeared as molecular peak plus 23. This happens because an adduct is formed between the compound and sodium. To avoid the adduct formation, water acidified with formic acid was added to the standards. The addition of acid breaks the adduct and allows to see the molecular peak.

Finally using the software from the MS-MS the optimization was done automatically, and a report was generated with the parameters that must be added to the method of analysis and the transitions of the 5 new compounds. Once the method was changed, an injection to the system was done to know the retention time (tr) of the compounds.

5.5. INSTRUMENTAL ANALYSIS

Instrumental analysis of OPFRs was carried out using turbulent flow chromatography - high pressure liquid chromatography - tandem mass spectrometry (TFC-HPLC-MS-MS), using the system Thermo Scientific TurboFlowTM, which allows to perform online sample purification and analysis. This system consisted of two C quaternary pumps and three LC columns, two of them for the purification and the last one for separation. The purification columns used were: CyclonTM-P (0.5 x 50 mm) and C18-XL (0.5 x 50 mm), and the chromatographic separation was done using the column Purosphere Star RP-18 (125 mm x 0.2 mm), which has a particle size of 5 µm. The tandem MS of this system was a triple quadrupole (QqQ) that uses heated-electrospray ionisation source (H-ESI).

The chromatographic separation was done using a flow rate of 0.25mL/min and the gradient can be found in manuscript of Giulivo, M., et al. [16]. The mobiles phases used for this separation are water with 0.1% of formic acid and methanol with ammonium acetate (10mM).

5.5.1. Turbulent flow chromatography

The part that can be more interesting about this way to analyse OPFRs is the TFC, because it allows to make the analysis without the need of making a previous purification to the injection into the chromatographic system.

TFC combines the size exclusion chromatography with the traditional stationary phase column chemistry, separating macromolecules from smaller molecules and the analytes of interest. Its main application is for on-line clean-up of biological matrices in LC-MS applications, reducing the time of sample preparation steps, which uses to be complex in biological matrices and reducing the sample preparation just to the extraction of the analytes. After injection into the system, the high flow rate, which uses to be between 1.5 and 5.0 mL/min, generates turbulent flow conditions into the column, making that the small molecules from the analyte become retained into the particle pores, while the macromolecules pass and go to waste. Once the analytes are extracted from the matrix, the elution starts moving them from the TFC column to the analytical column [17].

5.5.2. Analysed compounds

In *Table 2* there appear the compounds that were analysed with its corresponding retention time (tr), transition, the internal standard (IS) used for its quantification and the collision energy

that corresponds to each transition. As long as there do not exist a commercial IS for each compound, some of them are grouped using the IS that has a retention time more similar to it.

Table 2. Target compounds' and internal standards' parameters.

Compound	t_r	Transition	IS	Collision energy (V)
TCEP	4.65	287 → 99 287 → 224	1	38.6 15.6
TPPO	8.69	279 → 201 279 → 173	2	34.6 27.6
TClPP	9.43	327 → 99 327 → 250	2	23.6 9.6
TDClPP	11.14	431 → 99 431 → 320	2	26.6 16.1
TPHP	11.50	327 → 152 327 → 215	3	39.1 27.1
TNBP	12.45	267 → 99 267 → 210	4	20.1 7.6
DCP	12.65	341 → 151 341 → 228	4	35.6 27.1
TBOEP	12.94	399 → 299 399 → 199	5	17.1 13.1
TMCP	14.96	369 →165 369 → 91	5	58.6 41.1
EHDPP	15.68	363 → 251 363 → 152	5	44.6 14.6
IDPP	18.00	391 → 251 391 → 153	5	53.1 26.1
IPPP	19.75	453 → 327 453 → 369	5	29.1 26.6
THP	20.21	351 → 99 351 → 267	5	21.6 12.1
TEHP	28.97	435 → 80 435 → 99	5	58.6 36.1
Internal standards				
D12-TCEP	4.61	297 → 102 297 → 82	1	48.1 26.6
D15-TDClPP	11.07	445 → 102 445 → 331	2	27.6 13.6
D15-TPHP	11.38	342 → 160 342 → 223	3	37.6 28.1
D27-TNBP	12.25	294 → 102 294 → 82	4	58.1 28.1
13C2-TBOEP	12.94	404 → 302 404 → 98	5	31.6 14.6

5.6. QUALITY PARAMETERS

Quality parameters from applied methodology [16], are summarised in *Table 3*. The considered parameters were the recoveries (R) at low level (20 ng/g dw) and high level of concentration (100 ng/g dw), the reproducibility (evaluated with the RSD% in low and high concentration, using three replicates for each level of concentration), the method limits of detection (mLODs) and the method limits of quantification (mLOQs), where mLODs are calculated as signal to noise ratio (S/N) of 3 and the mLOQs in a S/N of 10. These parameters are the ones calculated for the fish matrix.

Table 3. Quality parameters.

Compound	Low level R (%)	RSD (%)	High level R (%)	RSD (%)	mLOD (ng/g lw)	mLOQ (ng/g lw)
TCEP	68	3.7	66	13	1.21	3.51
TPPO	47	2.5	51	7.4	0.35	1.30
TCIPP	64	3.0	61	2.4	1.48	4.18
TDCIPP	56	8.1	54	3.6	0.19	1.03
TPHP	53	2.5	52	7.3	1.30	3.45
TNBP	72	3.2	69	4.5	3.44	7.30
DCP	73	4	68	11	1.63	4.61
TBOEP	65	12	62	8.0	0.44	1.44
TMCP	78	13	76	9.1	2.55	4.63
EHDPP	62	16	58	3.6	0.53	0.97
IDPP	85	4.5	87	5.8	2.96	5.17
IPPP	82	9	80	12	19.3	24.8
THP	81	10	79	9.3	0.88	2.11
TPHP	98	12	96	10	1.95	3.86

6. RESULTS AND DISCUSSION

6.1. OPTIMIZATION OF MS-MS ANALYSIS FOR NEW OPFRS

Table 4 summarizes the values of tr obtained for the injection of the OPFRs, the two most intense transitions of each compound, the IS used for quantifying the compounds (chosen considering the most similar values of tr between the compound and the IS) and the collision energy of each transition. The IS numbers were specified previously in *Table 3*.

Table 4. New OPFRs optimization.

Compound	t_r	Transition	IS	Collision energy (V)
TEP	4.53	183 → 99 183 → 81	1	37 26
TPP	9.51	225 → 99 225 → 81	2	35 19
2IPPDPP	13.98	369 → 327 369 → 152	5	39 19
4IPPDPP	14.51	369 → 327 369 → 152	5	39 20
B4IPPPP	17.49	411 → 327 411 → 152	5	44 24

For the two isomers 2IPPDPP and 4IPPDPP, tr are sufficiently different to be able to proceed with their individual analysis and quantification.

In the case of these 5 OPFRs, quality parameters were not calculated, due to a lack of time to do the recovery tests, mLODs and mLOQs calculations. Therefore, the results obtained for these compounds will be considered as semiquantitative, because they will not be as reliable as the other 14 OPFRs which quality parameters were previously determined.

6.2. TURTLE SAMPLES

In the case of the turtles (Catalan and Balearic) the results were calculated in dry (dw) and wet weight (ww) because the lipid weight (lw) found in most of the turtles was too small, causing that the results in lw were too high. The percentage of lipids in turtles were between 0,28% and 52,7%, with a mean of 7,6%. As most values were really small it was considered a good option

to use ww instead of lw. In addition, the few published works evaluating the content of other families of POPs (PCBs, DDTs, …) in turtles also expressed the levels in wet weight instead of lipid weight [9].

6.2.1. Catalan coasts turtles

In the Catalan coasts turtles 13 of the total of 19 OPFRs were found and quantified, but not in all the samples. Two of the compounds (TCEP and TMCP) were detected in some cases but it was not possible to quantify them. This might be solved by expanding the calibration line in the low concentration points, but it was not possible as there was a lack of time.

The concentration levels of the 13 detected OPFRs are summarized in *Table 5*, considering the compound (Comp.), the maximum (Max.) and minimum (Min.) value found in the samples, the mean and the percentage of samples where it was found (% Det.). The concentrations are expressed in ng/g dw and ng/g ww. As replicates were not done there cannot be calculated means or RSD% for each individual.

Table 5. Concentration levels of OPFRs determined in Catalan coasts turtles.

Comp.	Min. (ng/g)		Max. (ng/g)		Mean (ng/g)		% det.
	dw	ww	dw	ww	dw	ww	
TEP	1.05	0.26	47.4	10.5	25.9	6.25	90
TPPO	0.031	0.007	3.07	0.78	0.64	0.16	85
TClPP	15.3	3.43	34.6	8.56	24.9	5.99	15
TPP	0.070	0.017	10.7	2.83	2.70	0.70	40
TDClPP	1.49	0.36	486	123	84.6	21.5	30
TPHP	1.63	0.37	198	50.5	52.2	13.2	20
TNBP	0.26	0.07	3.93	0.92	1.72	0.42	50
DCP	12.4	2.78	41.0	10.4	29.1	7.08	95
TBOEP	0.50	0.11	0.94	0.23	0.67	0.16	15
2IPPDPP	3.57	0.87	192	48.8	47.6	11.8	35
4IPPDPP	0.70	0.16	563	143	48.6	12.1	85
EHDPP	6.01	1.43	11.3	2.80	7.90	1.90	15
IPPP	38.3	9.47	49.4	12.0	43.8	10.4	15
∑OPFRs	48.0	12.6	1514	385	157	39.0	100

The concentration of each compound in each individual is shown in *Appendix 3* (*Table C*), with the concentrations in ng/g ww.

The most frequents OPFRs found in the Catalan coasts turtles are the DCP (95%), followed by TEP (90%), TPPO (85%) and 4IPPDPP (85%). The OPFRs that present higher levels are: TDClPP (mean value of 21.5 ng/g ww), TPHP (mean value of 13.2 ng/g ww), 4IPPDPP (mean value of 12.1 ng/g ww), 2IPPDPP (mean value of 11.8 ng/g ww) and IPPP (mean value of 10.4 ng/g ww).

6.2.2. Balearic coasts turtles

In the Balearic coasts turtles 14 of the total of 19 OPFRs were found and quantified, but not in all the samples. TCEP was detected in some cases but it could not be quantified, as happens in the Catalan coasts turtles, but just with this compound in this case.

The concentration levels of the 14 OPFRs are summarized in *Table 6*, following the same pattern that was applied for the Catalan coasts turtles.

Table 6. Concentration levels of OPFRs determined in Balearic coasts turtles.

Comp.	Min. (ng/g)		Max. (ng/g)		Mean (ng/g)		% det.
	dw	ww	dw	ww	dw	ww	
TEP	0.39	0.11	204	42.8	46.0	11.0	95
TPPO	0.092	0.017	1.17	0.37	0.47	0.13	95
TClPP	7.27	3.69	118	22.9	38.3	8.52	36
TPP	0.011	0.006	13.0	3.57	3.62	0.93	45
TDClPP	1.82	0.37	27.7	12.2	6.61	2.07	68
TPHP	0.43	0.087	5.54	2.20	3.22	0.88	41
TNBP	0.14	0.070	3.72	1.52	1.62	0.42	45
DCP	8.29	1.69	54.8	24.1	30.9	7.77	100
TBOEP	0.24	0.057	1.64	0.67	0.83	0.24	36
2IPPDPP	0.31	0.089	98.8	40.7	35.0	8.89	68
4IPPDPP	0.57	0.12	65.6	18.1	22.1	5.21	68
TMCP	15.9	3.83	97.0	23.8	48.5	11.8	15
EHDPP	0.43	0.081	8.83	2.51	3.48	1.09	27
IPPP	33.4	7.78	142	27.6	60.1	12.7	23
∑OPFRs	23.5	12.0	320	100	157	38.3	100

The concentration of each compound in each individual is shown in *Appendix 4* (*Table D*), with the concentrations in ng/g ww.

In this case, the most common OPFRs found in the turtles are the DCP (100%) followed by TEP (95%) and TPPO (95%). And the ones showing higher concentration levels are: IPPP

(mean value of 12.7 ng/g ww), TMCP (mean value of 11.8 ng/g ww), TEP (mean value of 11.0 ng/g ww), 2IPPDPP (mean value of 8.89 ng/g ww) and TClPP (mean value of 8.52 ng/g ww).

6.2.3. Comparison of turtle samples

The OPFRs found in both type of turtles are relatively similar, in both cases DCP, TEP and TPPO were ones of the most common ones, but the levels found in them are more different. There are only two OPFR that have a high concentration in both cases, which are the IPPP and 2IPPDPP, the rest of them are relatively different.

In this box plot (*Figure 5*) are represented the values of ∑OPFRs for Catalan coasts and Balearic coasts turtles, in ng/g dw and ng/g ww. For the ∑OPFRs of Catalan coasts turtles an outlier has been eliminated, because it had a value of 1515 ng/g dw because the boxplot got distorted. The sample was also eliminated in ww values.

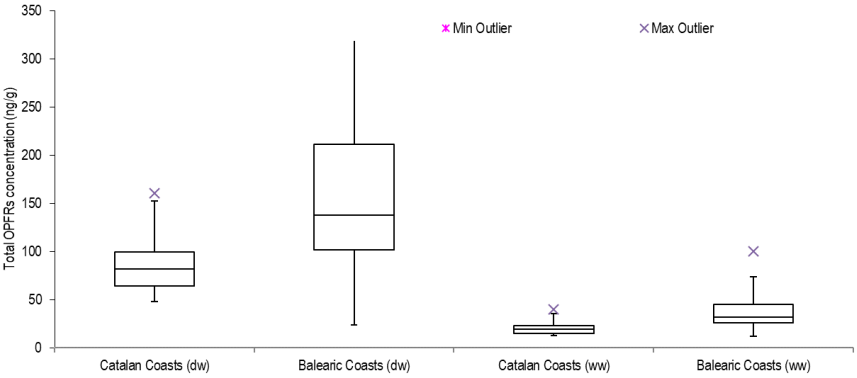


Figure 5. Box plot graphic of ∑OPFRs in Catalan and Balearic coasts turtles.

As it can be seen in the boxplot, it seems that the levels of OPFRs in the Balearic coasts turtles are slightly higher than the ones found in Catalan coasts turtles. Also, the variability between individuals is higher in the Balearic coasts turtles than in the Catalan ones.

In order to see if the levels of OPFRs in Balearic turtles significantly differed from the Catalan turtles, statistical tests were performed.

First of all, a F-test was performed to check if the variance of Balearic turtles was higher than the Catalan turtles one, to know which kind of Student's t-test perform, equal or unequal variances. The values obtained in the F-test considering a significance level of 0.05 were:

F_{cal}=14.15 and F_{tab}=2.11, meaning that the null hypothesis was rejected as the F_{cal} value was higher than the F_{tab} value, and it can be said that the variance of Balearic turtles is significantly higher than the Catalan ones.

As a result of the F-test, a Student's t-test for unequal variances was performed to the two group of samples, in order to know if the means are significantly equal or the Balearic turtles one was higher than the Catalan turtles one. The significance level chosen was also 0.05, and the results obtained were: t_{cal}=0,03 and t_{tab}(1 tail)=1,72. As the value of t_{cal} is smaller than the t_{tab} one, the null hypothesis is accepted, and it can be said that the means of the two groups of turtles are not significantly different.

In order to compare the total OPFRs found with the obtained values from other studies, it will be compared with other POPs as ∑PCBs and ∑DDTs, which can be found in different references. The values of ∑PCBs found in other studies were around 0.6 and 23.5 ng/g ww (in muscle) [10], and 551 (473) ng/g ww or 256 (269) ng/g ww (mean in brackets) depending on the type of turtle [9]. ∑DDTs values are slightly smaller, going from 0.3 to 4.9 ng/g ww in muscle [9].

The obtained values in this study of ∑OPFRs go from 12.6 to 385 ng/g ww in Catalan coasts turtles, with a mean of 39.0 ng/g ww and from 12.0 to 100 ng/g ww in the Balearic coasts turtles, with a mean of 38.3 ng/g ww. These values can be compared to the ones found in the previously mentioned studies for persistent organic pollutants, they seem to be slightly higher than the first value of ∑PCBs but they are in the ranges from the second article ([9]). Comparing with ∑DDTs the values of ∑OPFRs are higher than the ones previously mentioned.

6.3. FISH SAMPLES

In the fish samples 8 of the total of 19 OPFRs were found and quantified, but not in all samples. That was mainly because the chromatograms were not as clear as in the turtle case, probably because the liver matrix cannot be completely purified using the TFC methodology. This might be solved by doing some pre-treatment to the samples, apart from the extraction, in order to purify it before the injection.

In this case, the lipidic content of all the samples was high enough to allow the expression on the results in lw, it was between 6,6% (minimum) and 64,4% (maximum), with a mean of 32,9%. Even if the results were not good it would have not been possible to show the results in ww because the samples were given lyophilized, so the percentage of water in the sample is

unknown. The concentration levels of OPRFs that were determined in the fishes samples are summarized in *Table 7*.

Table 7. Concentration levels of OPFRs determined in fishes.

Comp.	Min. (ng/g)		Max. (ng/g)		Mean (ng/g)		% det
	dw	lw	dw	lw	dw	lw	
TEP	2.19	5.13	14.6	88.3	6.38	31.4	50
TPPO	67.9	114	83.3	768	74.8	261	64
TPHP	0.48	0.83	51.2	79.4	6.55	18.2	86
DCP	21.7	81.2	57.3	702	41.0	331	50
2IPPDPP	5.43	8.43	5.43	8.43	5.43	8.43	14
4IPPDPP	66.1	102	66.1	102	66.1	102	14
TMCP	4.08	9.49	24.0	255	15.3	87.0	79
IPPP	44.9	79.4	277	703	83.3	343	57
∑OPFRs	10.4	83.5	519	2159	132	591	100

The concentration of each compound in each individual is shown in *Appendix 4* (*Table E)*, with the concentrations in ng/g lw.

In the fish samples the most frequent OPFRs are: TPHP (86%) and TMCP (79%). The ones that show a higher levels are IPPP (mean value of 343 ng/g lw) and DCP (mean value of 331 ng/g lw), followed by TPPO (mean value of 261 ng/g lw).

The OPFRs found in fish samples are different than the ones found in turtles, TPHP and TMCP that are the most common in the fish samples, were only found in some turtle samples. TPHP was found in 20% of the Catalan coasts turtles, and in 41% of the Balearic ones. TMCP that was found in a 79% of the fish samples was just found in a 15% of the Balearic coasts turtles, but in none of the Catalan ones.

In order to compare the values of ∑OPFRs with other studies, it will be compared with a study in freshwater fish [11], as long as there were not found studies in marine fish. The values of ∑OPFRs that were found vary depending on the sampling place, in Evrotas river basin they went from 34.1 to 55.5 ng/g lw with a mean of 40.1 ng/g lw, in Adiege river basin go from 50.6 to 650 ng/g lw with a mean of 286 ng/g lw and in Sava river basin go from 14.4 to 196 ng/g lw with a mean of 84 ng/g lw. It can be seen that the values found in the current study of marine fishes are higher than the ones in freshwater fish, as long as the values go from 83.5 to 2159 ng/g lw with a mean of 591 ng/g lw. The marine fishes seem to be more polluted than the freshwater ones, but it must be considered that the freshwater fishes are smaller than the marine ones.

While freshwater weigh was between 200-500 g the marines that have been studied can weigh up to 12 kg.

10. CONCLUSIONS

The TFC-HPLC-MS-MS analysis of 5 additional OPFRs was optimized with satisfactory results in the calibration curve and in the determination of these compounds in biota samples. The main problem was that the quality parameters could not be established, and the results cannot be considered quantitative, but only semiquantitative. This can be solved in the future by making the recovery test and calculating mLODs and mLOQs, to confirm that the compounds are well extracted and well recovered. That would increase the total amount of quantifiable OPFRs.

The levels of OPFRs obtained in the current study are in the same order of magnitude than other contaminants as PCBs or DDTs that were found in previous studies in turtles. This can indicate that OPFRs are present as other contaminants that nowadays are more regulated. This might be due to the amount of debris that is throwed to oceans, and this compounds bioaccumulate in biota.

Balearic coasts turtles seem to be more polluted that the ones from Catalan coasts. Even if the means are significantly equal, the dispersion of results is higher in the Balearic coasts turtles than in the Catalan coasts ones.

The marine fish samples have higher concentrations than those found in other studies performed with freshwater fish. This might be due to the difference in fish size and in trophic level of studied fishes, as the marine were bigger than the freshwater and thus, they would be able to bioaccumulate higher amounts of OPFRs.

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12. ACRONYMS

- CCL: Curve carapace length
- dw: Dry weight
- FL: Fork length
- FR: Flame retardant
- HPLC: High performance liquid chromatography
- IS: Internal standard
- lw: Lipid weight
- mLOD: Method limit of detection
- mLOQ: Method limit of quantification
- MS-MS: Tandem mass spectrometry
- OPFR: Organophosphorus flame retardant
- TFC: Turbulent flow chromatography
- ww: Wet weight

APPENDICES

APPENDIX 1: SAMPLE INFORMATION

Table A. Turtle size information.

Catalan Coasts			Balearic Coasts		
Year	**ID**	**CCL (cm)**	**Year**	**ID**	**CCL (cm)**
2014	CC11	41	2014	CB2	77
2015	CC4	57	2014	CB3	62
2015	CC5	59	2014	CB18	66
2015	CC16	54	2014	CB21	26
2015	CC17	30.6	2014	CB22	43
2015	CC22	50	2014	CB23	61
2015	CC23	68	2015	CB12	16
2016	CC6	35	2015	CB19	52
2016	CC9	43	2016	CB1	57
2016	CC12	46	2016	CB7	69
2016	CC13	34	2016	CB10	38
2016	CC14	58.5	2016	CB11	39.5
2016	CC20	68	2016	CB13	70
2017	CC3	61	2016	CB20	60
2017	CC7	58	2016	CB16	65
2017	CC8	78	2017	CB5	24.5
2017	CC10	36	2017	CB6	52
2017	CC15	45	2017	CB8	24.5
2017	CC18	48	2017	CB9	48
???	CC21	44.7	2017	CB14	42
			2017	CB15	47
			2017	CB17	60

Table B. Fish weight and sex information.

Code	Weight (g)	Sex
LTA16/001	2788	2
LTA16/002	3196	1
LTA16/003	2668	2
LTA16/004	3844	2
LTA16/005	2780	2
LTA16/006	12000	2
LTA16/007	8486	1
LTA16/008	10474	2
LTA16/009	7954	2
LTA16/010	9114	1
LTA16/011	8198	1
LTA16/013	9906	1
LTA16/014	9784	2
LTA16/015	8602	2

APPENDIX 2: CATALAN COASTS LEVELS

Table C. OPFRs concentration levels for each sample (Catalan Coasts).

ng/g ww

	TEP	TPPO	TClPP	TPP	TDClPP	TPHP	TNBP
CC3	7.02	0.78	nd	0.71	123.72	*50.49*	nd
CC4	6.76	0.05	nd	0.25	nd	nd	nd
CC5	*4.59*	0.20	nd	nd	nd	nd	*0.73*
CC6	5.09	nd	nd	nd	nd	nd	nd
CC7	*6.05*	0.33	nd	nd	nd	nd	nd
CC8	*0.26*	0.37	nd	*0.02*	*1.13*	1.01	*0.23*
CC9	5.12	0.06	nd	2.83	nd	nd	nd
CC10	4.13	0.06	nd	nd	nd	nd	nd
CC11	5.25	0.02	nd	nd	0.36	nd	nd
CC12	6.45	nd	nd	nd	nd	nd	nd
CC13	10.5	0.01	nd	nd	nd	nd	*0.92*
CC14	*5.13*	0.14	nd	nd	nd	nd	*0.25*
CC15	nd	0.04	*3.43*	nd	*0.60*	0.37	*0.23*
CC16	6.23	nd	nd	nd	nd	nd	*0.07*
CC17	8.87	0.10	nd	nd	nd	nd	*0.37*
CC18	nd	0.02	*8.55*	*0.03*	*2.37*	1.09	*0.09*
CC20	4.87	0.09	nd	1.35	nd	nd	nd
CC21	10.5	0.10	nd	0.16	0.57	nd	nd
CC22	9.21	0.09	nd	nd	nd	nd	*0.89*
CC23	6.60	0.23	nd	0.23	nd	nd	*0.42*

Table C (*continued*). OPFRs concentration levels for each sample (Catalan Coasts).

ng/g ww

	DCP	TBOEP	2IPPDPP	4IPPDPP	EHDPP	IPPP	ΣOPFRs
CC3	10.4	nd	*48.8*	*143*	nd	nd	**385**
CC4	6.95	nd	nq	*4.23*	nd	nd	**18.2**
CC5	8.44	nd	nd	nd	nd	nd	**14.0**
CC6	7.20	nq	nq	*6.95*	nd	nd	**19.2**
CC7	7.45	nd	nd	*2.11*	nd	nd	**15.9**
CC8	4.79	0.23	*0.87*	nd	*1.46*	*12*	**22.4**
CC9	8.41	nd	nq	*5.14*	nd	nd	**21.6**
CC10	6.25	nd	nq	*3.74*	nd	nd	**14.2**
CC11	8.13	nd	*8.25*	*4.08*	nd	nd	**26.1**
CC12	6.21	nd	*3.73*	*4.61*	nd	nd	**21.0**
CC13	7.28	nd	nq	*0.83*	nd	nd	**195**
CC14	7.84	nd	nd	*0.34*	nd	nd	**13.7**
CC15	2.78	0.11	*1.46*	*0.16*	*1.43*	*9.80*	**20.4**
CC16	6.61	nd	nq	nd	nd	nd	**12.9**
CC17	nd	nd	nq	*3.25*	nd	nd	**12.6**
CC18	6.46	0.14	nd	*0.72*	*2.80*	*9.47*	**31.7**
CC20	8.07	nd	nq	*3.64*	nd	nd	**18.0**
CC21	6.28	nd	*1.80*	*9.10*	nd	nd	**28.5**
CC22	6.97	nd	nq	*7.05*	nd	nd	**24.2**
CC23	7.92	nd	*17.8*	*6.41*	nd	nd	**39.7**

The results shown in cursive have a value of RSD% higher than 30% when it is calculated comparing the calibration line area relation (first transition versus second transition) with the compound area relation. Even if they cannot be considered as quantitative results because it is not possible to confirm that it is the same compound, they have been reported in order to have an idea about the possible levels. This has been done for all three type of samples (Catalan coasts turtles, Balearic coasts turtles and fish).

APPENDIX 3: BALEARIC COASTS LEVELS

Table D. OPFRs concentration levels for each sample (Balearic Coasts).

ng/g ww

	TEP	TPPO	TCIPP	TPP	TDCIPP	TPHP	TNBP	DCP
CB01	4.28	0.02	nd	1.91	nd	nd	nd	*10.2*
CB02	9.59	nd	nd	nd	nd	nd	*0.48*	8.66
CB03	*1.74*	0.02	*5.86*	nd	*0.76*	0.60	*0.41*	2.77
CB05	42.8	0.07	nd	0.43	0.53	nd	nd	8.12
CB06	*0.11*	0.15	*4.77*	nd	*0.37*	0.09	*0.41*	1.69
CB07	22.3	0.08	nd	2.71	nd	nd	nd	*10.9*
CB08	*2.94*	0.11	*8.67*	*0.06*	*0.73*	0.43	*0.45*	2.77
CB09	7.17	0.05	nd	nd	0.73	nd	nd	*7.13*
CB10	21.0	0.10	nd	nd	nd	nd	nd	6.85
CB11	10.1	0.06	nd	0.15	0.83	nd	nd	*9.93*
CB12	nd	0.10	*22.9*	nd	*0.37*	1.00	*0.36*	7.22
CB13	*1.30*	0.12	*6.52*	*0.01*	*1.33*	0.58	*0.14*	3.75
CB14	*2.48*	0.33	nd	nd	*1.82*	0.90	*0.07*	6.41
CB15	18.0	0.04	nd	nd	nd	nd	nd	7.69
CB16	14.1	0.05	nd	nd	nd	nd	nd	*6.17*
CB17	*0.41*	0.17	*10.4*	*0.06*	*1.56*	1.33	*0.25*	8.50
CB18	5.31	0.03	nd	nd	0.70	nd	nd	7.11
CB19	15.5	0.17	nd	nd	12.2	nd	nd	*24.1*
CB20	*0.20*	0.25	*3.69*	0.01	1.39	0.79	*0.07*	4.80
CB21	*12.8*	0.37	*5.39*	*0.38*	*2.64*	2.20	*1.52*	10.6
CB22	17.6	0.09	nd	nd	nd	nd	nd	7.80
CB23	*20.4*	0.32	nd	3.57	*5.11*	nq	nd	7.80

Table D (*continued*) . OPFRs concentration levels for each sample (Balearic Coasts).

ng/g ww

	TBOEP	2IPPDPP	4IPPDPP	TMCP	EHDPP	IPPP	ΣOPFRs
CB01	nd	nd	5.51	*23.8*	nd	nd	**45.7**
CB02	nd	*8.81*	*1.81*	nd	nd	nd	**29.4**
CB03	0.08	*3.46*	*0.44*	nd	*0.08*	*8.94*	**25.2**
CB05	nq	*3.89*	*6.36*	nd	nd	nd	**62.2**
CB06	0.06	*1.18*	*0.12*	nd	*0.27*	7.78	**17.0**
CB07	nq	*2.51*	*9.28*	3.83	nd	nd	**51.6**
CB08	nd	nd	nd	nd	nq	nd	**16.2**
CB09	nd	*5.69*	*9.59*	nq	nd	nd	**30.4**
CB10	nd	*4.85*	nd	nq	nd	nd	**32.8**
CB11	nd	nq	1.65	7.91	nd	nd	**30.6**
CB12	0.28	*1.98*	*0.30*	nd	nq	*27.6*	**62.1**
CB13	0.40	nd	nd	nd	nq	nd	**14.2**
CB14	0.20	*0.09*	nd	nd	*2.51*	*12.0*	**25.8**
CB15	nd	*4.28*	*1.60*	nq	nd	nd	**31.6**
CB16	nd	*5.17*	*1.49*	nd	nd	nd	**27.0**
CB17	0.07	nd	nd	nd	*0.95*	*8.04*	**31.7**
CB18	nd	*16.1*	*6.61*	nd	nd	nd	**35.9**
CB19	nd	*40.7*	*7.86*	nq	nd	nd	**100**
CB20	*0.12*	nd	nd	nd	*0.64*	nd	**12.0**
CB21	0.67	nd	nd	nd	*2.10*	nd	**38.6**
CB22	nd	*11.0*	*7.45*	nq	nd	nd	**43.9**
CB23	nd	*23.7*	*18.1*	nq	nd	nd	**79.0**

APPENDIX 4: FISH LEVELS

Table E. OPFRs concentration levels for each sample (fish).

	ng/g lw								
	TEP	**TPPO**	**TPHP**	**DCP**	**2IPPDPP**	**4IPPDPP**	**TMCP**	**IPPP**	**ΣOPFRs**
LTA 16 001	nd	nq	nq	nd	nq	nq	*125*	nq	**125**
LTA 16 002	*30.8*	nq	*22.6*	*168*	nq	nq	*81.0*	nq	**303**
LTA 16 003	*88.3*	nq	*32.1*	*702*	nq	nq	*255*	703	**1781**
LTA 16 004	*10.5*	nq	*5.64*	*238*	nq	nq	*112*	*242*	**610**
LTA 16 005	nq	768	*30.7*	*615*	nq	nq	*175*	*569*	**2159**
LTA 16 006	nd	119	nq	nd	nq	nq	nd	nd	**119**
LTA 16 007	nd	287	6.02	*81.2*	nq	nq	*53.3*	*167*	**595**
LTA 16 008	nd	127	*3.86*	nd	nq	nq	*40.9*	nd	**171**
LTA 16 009	*30.8*	nq	nq	nd	nq	nd	*52.7*	nd	**83**
LTA 16 010	*22.7*	124	79.4	nd	*8.43*	*102.62*	*37.2*	*431*	**805**
LTA 16 011	nd	nd	1.28	nd	nq	nq	nd	*166*	**167**
LTA 16 013	nd	157	*9.51*	nd	nq	nq	*9.49*	nd	**176**
LTA 16 014	5.13	114	0.83	nd	nq	nd	*14.3*	*79.4*	**213**
LTA 16 015	nd	391	8.05	*183*	nq	nd	nd	*384*	**967**